Available online at www.sciencedirect.com
SciVerse ScienceDirect
Procedia Food Science 1 (2011) 7 – 11
Procedia Food Science

11th International Congress on Engineering and Food (ICEF11)

Morphology and fluorescence properties of dye-entrapped silica nanoparticles

Namsoo Kim *, Sun-Mi Oh, Chong-Tai Kim, Chul-Jin Kim, Yong-Jin Cho

Food Bio-nano Technology Research Group, Korea Food Research Institute, Baekhyun 516, Bundang, Songnam, Republic of Korea

Abstract

Dye-entrapped silica nanoparticles can be used to label biological components in immunosensing since they are bright and photostable, less prone to stochastic blinking with sustained imaging, biologically inert and thus basically non-toxic, and compatible with aqueous system due to hydrophilic surface. Therefore, the objective of this study was to produce dye-entrapped silica nanoparticles and to determine the morphological and spectral characteristics of these particles. Silica spheres were produced by a base-catalyzed entrapment protocol, and were determined for their properties by particle size analysis, SEM imaging, and analyses for fluorescence emission spectra and fluorescence kinetics. By the above procedure, dichlorotris(1,10-phenanthroline)ruthenium(II) (Ruphen) was entrapped into the silica matrix successfully. However, the incorporation of fluorescein, rhodamine B and 5(6)-carboxytetramethyl-rhodamine was not possible. The yield, particle size and shape of the Ruphen silica nanoparticles were dependent upon reaction variables. Compared to free dye, the silica spheres generally showed higher fluorescence intensity and stability, which seemed to be very advantageous for a sensitive biomarker detection needed to evaluate food functionality.

Selection and/or peer-review under responsibility of 11th International Congress on Engineering and Food (ICEF 11) Executive Committee.

Keywords: morpholpgy; fluorescence properties; dye-entrapped; silica nanoparticles

1. Introduction

The importance of biomarker proteins related with a disease or metabolic syndrome in the blood of a model animal or human has been increasing in recent years for the dual purposes of diagnosis and homogeneous evaluation of food functionality. The detection of serum biomarker proteins has been conventionally carried out by HPLC, spectrophotometry and enzyme-linked immunosorbent assay. With the advent of efficient fluorescence labels, fluoroimmunosensing and fluoroimmunoassay based on the

* Corresponding author. Tel.: +82-31-780-9131; fax: +82-31-709-9876.
E-mail address: *k9130sen@hanmail.net*

Selection and/or peer-review under responsibility of 11th International Congress on Engineering and Food (ICEF 11) Executive Committee.
doi:10.1016/j.profoo.2011.09.002

complex formation between antigen and antibody seems to be promising for biomarker detection due to its intrinsic sensitivity, specificity and rapidity.

Fluorescent silica nanoparticles (FSNPs) could be key nano-materials to label antibody or antigen for immunosensing since they are bright and photostable, less prone to stochastic blinking with sustained imaging, appropriate for size-selective analysis, biologically inert and thus potentially non-toxic, compatible with aqueous system due to silica surface, and suitable for multiple coding analyses. These properties make an optoelectronic immunosensor that exploits FSNP fluorescence extremely useful for the detection of serum biomarker proteins [1,2].

We have studied on various FSNPs that are manufactured by dye entrapment and core-shell procedure, and succeeded in producing silica nanoparticles that showed wide size distribution. By using these silica particles, we are going to develop highly sensitive fluorescence-based methods of immunosensing and immunoassay to detect biomarker proteins. In this study, we first report the morphology and fluorescence properties of the dye-entrapped silica nanoparticles that we have made with comparison to those of corresponding free dye.

2. Materials and Methods

2.1. Fluorescent dyes and FSNP preparation

The fluorescent dyes that were used to manufacture FSNPs comprised dichlorotris(1,10-phenanthroline)- ruthenium(Ⅱ) hydrate (Ruphen), 5(6)-carboxytetramethylrhodamine (CTMR), fluorescein and rhodamine B. The chemical structures of these dyes are shown in Fig. 1.

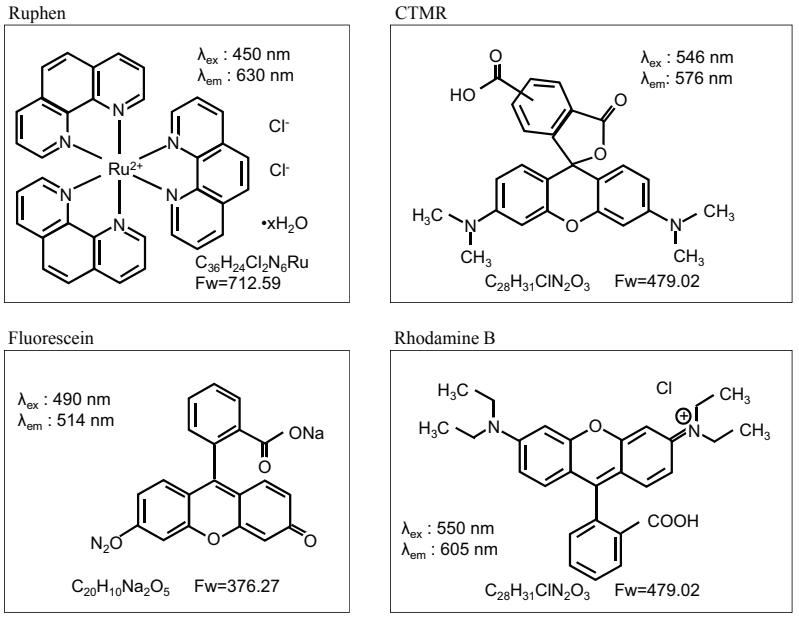

Fig. 1. Chemical structures of fluorescent dyes used to manufacture silica nanoparticles.

The dye-entrapped silica nanoparticles were individually prepared according to Stöber method [3] with some modification (Fig. 2).

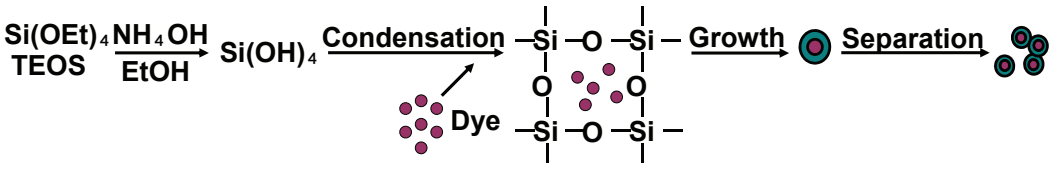

Fig. 2. Schematic representation to manufacture dye-entrapped silica nanoparticles.

2.2. Characterization of FSNPs

The particle size of the silica nanoparticles in aqueous status was determined with particle size analyzer (Nanotrac™ 250, Microtrac, USA) and the surface morphology of them in dry status was imaged with ultra high resolution scanning electron microscopy (UHR-FE-SEM, S-4800, Hitachi, Japan). Fluorescence emission spectra and fluorescence kinetics were measured with microplate reader (Spectramax® M2e, Molecular Devices, USA). The detection of fluorescence images of the silica nanoparticles was carried out with a fluorescence microscope for Epi-fluorescence measurement (Nikon Eclipse 80i, Nikon Inc., Melville, NY, USA). The images were collected through a 20× microscope objective using a Epi-fluorescence filter block N B-2A containing 450-490 nm band pass excitation filter, a 505 nm dichroic mirror and a 520 nm barrier filter. A monochrome cooled digital camera head DS-Qi1 (Nikon Inc.) was used for digital imaging.

3. Results and Discussion

The silica nanoparticles were prepared by the individual entrapment of Ruphen, fluorescein, rhodamine B and CTMR. When they were analyzed for particle size, fluorescence images and SEM images, a unique feature was obtained. According to the results of particle size analysis and SEM images, the silica particles prepared by the individual addition of fluorescein, rhodamine B and CTMR were formed very well. However, the fluorescent particles were not found in fluorescence imaging (Table 1, Fig. 3). This fact indicated that fluorescein, rhodamine B and CTMR were not entrapped inside silica particles. On the contrary, the silica particles prepared by the addition of Ruphen showed well dispersed fluorescent particles in fluorescence imaging. We presumed that some factors such as dye molecular weight might contribute to this phenomenon.

Table 1. Particle size and output of silica particles prepared by dye entrapment procedure

Dye	Output (mg)	Particle size (nm)
Ruphen	402.2	687.4 ± 27.7 [a]
Fluorescein	403.9	624.9 ± 329.8
Rhodamine B	326.7	233.6 ± 7.0
CTMR	418.3	763.6 ± 61.1

[a] Mean±SD (n=5).

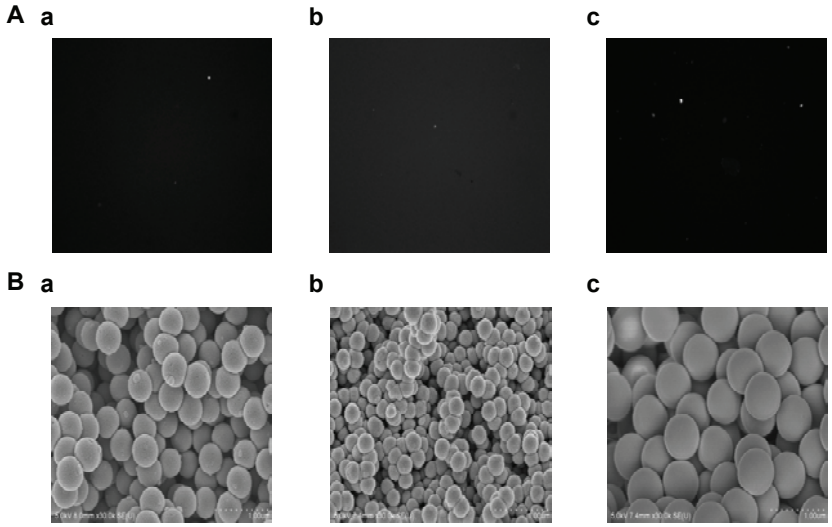

Fig. 3. Fluorescence and SEM images of silica nanoparticles entrapped with fluorescein, rhodamine B and CTMR. Panels A and B indicate fluorescence and SEM images, respectively. Fluorescent dyes used for entrapment procedure : a, fluorescein; b, rhodamine B; c, CTMR.

The fluorescence properties of Ruphen FSNP that showed the fluorescent particles in fluorescence imaging were analyzed to obtain fluorescence emission spectra and fluorescence kinetics. The Ruphen FSNP showed the higher fluorescence intensity at the emission maximum just below 600 nm compared to that of the corresponding free dye. The FSNP also showed a good photo-stability compared to the free dye counterpart.

4. Conclusion

Out of the tested fluorescent dyes, Ruphen only was entrapped into the silica matrix. The Ruphen FSNP seemed to be ideal to conduct sensitive biomarker detection because of its higher fluorescence intensity and stability.

Acknowledgements

This study was carried out as one part of the research project of Development of Food Nanotechnology, Korea Food Research Institute, Republic of Korea.

References

[1] Rossi LM, Shi L, Rosenzweig N, Rosenzweig, Z. Fluorescent silica nanospheres for digital counting bioassay of the breast cancer marker HER 2/nue. *Biosens Bioelectron* 2006;**21**:1900-1906.

[2] Choi J, Burns AA, Williams RM, Zhou Z, Flesken-Nikitin A, Zipfel WR, Wiesner U, Nikitin AY. Core-shell silica nanoparticles as fluorescent labels for nanomedicine. *J Biomed Opt* 2007;**12**:064007

[3] Stőber W, Fink A, Bohn E. Controlled growth of monodisperse silica spheres in the micron size range. *J Colloid Interf Sci* 1968; **26**: 62-69.

Presented at ICEF11 (May 22-26, 2011 – Athens, Greece) as paper FMS186.